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CRYPTOSPORIDIOSIS IN IMMUNOCOMPROMISED PATIENTS IN A TURKISH UNIVERSITY HOSPITAL

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The prevalence of Cryptosporidiosis in 18 immunosuppressed diarrheic patients was evaluated by examination of fecal samples by direct staining (Modified Kinyoun and Giemsa), direct and indirect immunofluorescence methods. Forty patients (10 nondiarrheic immunosuppressed, 10 nondiarrheic immunocompetent, and 20 diarrheic immunocompetent) were included in the study as the control group. 11 of 18 samples were positive for cryptosporidial oocysts by at least one of the methods. Oocysts were detected in all (n=7) of the AIDS patients. This high frequency was attributed to a probable nosocomial infection. None of the samples from control subjects were found positive for Cryptosporidium. Our results indicate that Cryptosporidial oocysts should be detected particularly in immunosuppressed patients with diarrhea. Modified Kinyoun staining method is practical and reliable for this purpose. Immunofluorescence staining methods can be applied for confirmation of the results.

Cryptosporidium parvum is a coccidian protozoan, which has recently been recognized to be a human pathogen. The parasite has been detected to be nestled between the microvilli of gastrointestinal and respiratory epithelial cells. Cryptosporidial oocysts can be transmitted from human-to-human and animal-to-human via oral-fecal route by consumption of contaminated water and raw milk. Day-care centers and nosocomial outbreaks highly facilitate person-to-person spread [1–3].

Cryptosporidiosis is more commonly encountered in malnourished children, elderly and immunocompromised patients in underdeveloped countries [4]. While diarrhea is self-limited and lasts not more than 1-2 weeks in immunologically intact hosts, the clinical course is life-threatening in immunocompromised individuals. Profuse and long-lasting diarrhea, dehydration, electrolyte imbalance and extraintestinal invasion is met particularly in patients with AIDS [5, 6].

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The present study was undertaken to determine the prevalence of Cryptosporidiosis in immunocompromised patients with diarrhea and comparatively evaluate the use of various methods in detection of Cryptosporidium oocysts in fecal samples of the corresponding patients.

Materials and methods

Patients and control subjects. The study group consisted of 18 patients who were immunosuppressed due to various disorders and/or immunosuppressive drugs. Fecal samples were sent to Parasitology Laboratory, Department of Microbiology, Hacettepe University in a period of 17 months between July 1995 and January 1997 for detection of Cryptosporidium and other parasites. Forty patients constituted the control subjects of the study; these were chosen among immunosuppressed non-diarrheic patients (n=10), immunocompetent subjects who were examined in the hospital for signs and symptoms other than diarrhea and whose fecal samples were referred to Parasitology Laboratory for routine examination (n=10) and immunocompetent subjects who were admitted for diarrhea (n=20). Characteristics of the patients and the control subjects enrolled in the study are shown in Table I.

Fecal sample. The samples were stored at –30 °C in 10% formalin solution until tested and centrifuged prior to processing. Three samples were collected from each patient.

Detection of cryptosporidial oocysts

Direct staining methods. Fecal smears, stained by Modified Kinyoun Acid-Fast (MAF, Figure 1) and Giemsa method were examined under light microscope and Cryptosporidial oocysts were defined as described previously [7].

Direct fluorescence antibody stain (DIF). The method was performed by a commercially available "Merifluor Cryptosporidium Giardia kit" (Meridian Diagnostics, Inc. Ohio 45244) and the results were evaluated by fluorescence microscopy. Application of the test and interpretation of the results were done according to the instructions of the manufacturer [8, 9].

Indirect fluorescence stain (IIF). Mouse monoclonal antibodies (IgG and IgM culture supernatants) specific to *Cryptosporidium* oocysts were kindly provided from San Francisco General Hospital, Division of Infectious Diseases. The test was employed according to the instructions of "Merifluor Cryptosporidium indirect immunofluorescence kit" (Meridian Diagnostics, Inc. Ohio 45244) and the results were interpreted under fluorescence microscope, as described previously [8, 9].

Routine parasitological examination. Fecal samples of all patients included in the study were examined for the probable existence of egg and/or cyst form of commonly encountered parasites other than Cryptosporidium, by standard methods [10].

Results

Cryptosporidial oocysts could be detected in fecal samples of 11 out of 18 immunosuppressed diarrheic patients with at least one of the methods included in the study. Both direct staining and direct and indirect immunofluorescence methods were positive for 6 of 11 patients. Oocysts were detected by direct staining and direct immunofluorescence tests in 4 patients. The fecal sample of the remaining patient revealed the presence of oocysts by immunofluorescence methods only. All AIDS patients (n=7) were found to carry Cryptosporidial oocysts in faeces by at least one of the methods

Table I

Characteristics of patients enrolled in the study

Characteristic	Value
Patients	
No. of patients (M/F)	18 (10/8)
Age range in years (mean)	24–56 (39.7)
No. with immunosuppression and diarrhea	18
AIDS	7
AML	5
NHL	2
ALL	1
SLE	1
RT	1
Myelofibrosis	1
Control subjects	
No. of patients (M/F)	40
Age range in years (mean)	17–62 (36.6)
No. with immunosuppression and without diarrhea	10
AML	4
RT	3
ALL	1
NHL	1
HL	1
No. without immunosupression and without diarrhea	10
No. without immunosupression and with diarrhea	20

AML: Acute myeloblastic leukemia
ALL: Acute myeloblastic leukemia
NHL: Non-Hodgkin lymphoma
HL: Hodgkin lymphoma
SLE: Systemic lupus erythematosus
RT: Renal transplantation

used in the study. Time for hospitalization and detection of cryptosporidial oocysts in stool samples of AIDS patients were as follows: June 1995 (n=2), January 1996 (n=1), March 1996 (n=1), April 1996 (n=2), and May 1996 (n=1). All of the patients were hospitalized in AIDS Unit. Table II summarizes the clinical features and results of the Cryptosporidial oocyst detection tests of the 11 patients with cryptosporidiosis.

Cryptosporidial oocysts could be detected in none of the samples obtained from the control subjects whereas routine parasitological examination of these samples yielded the presence of Giardial cysts in two.

Discussion

The role of *Cryptosporidium parvum* in life-threatening diarrhea particularly in patients whose immune system has been suppressed due to various disorders or iatrogenic reasons is now well-established [5]. Detection of the definitive agent of diarrhea not only facilitates the direction of appropriate therapeutic approach but also avoids the use of unnecessary treatment modalities. The significance of the entity is more emphasized, depending on the data showing that the mortality in AIDS patients is due to opportunistic infections in about 90% of the cases. Cryptosporidiosis has been defined to cause a seriously debilitating clinical picture characterized by cholera-like profuse diarrhea, dehydration and electrolyte imbalance in patients with AIDS. Since asymptomatic carriage state has been reported in HIV-infected persons, it has been recommended that the fecal samples of such patients should be examined for detection of Cryptosporidial oocysts even in the absence of gastrointestinal symptoms [11–13].

Fig. 1. Cryptosporidium oocysts by MAF (×1000)

Table II

Clinical features and the results of direct staining and immunofluorescence methods of the patients who were found positive for Cryptosporidium

Case No.	Age	Gender	Underlying disorder	Clinical features	Giemsa	MAF	DIF	IIF
1 NÖ	29	F	AIDS (heterosexual transmission)	Watery, profuse diarrhea for 4–5 months, 4–5 times/day	±*	+	±	–
2 FN	35	M	AIDS, PCP (heterosexual transmission)	Watery, profuse, mucus containing diarrhea for one year, 10 times/day	+	+	+	+
3 HÜ	42	M	AML, BMT	Watery, mucus containing diarrhea for 2 days, 2–3 times/day	±	+	+	+
4 GE	24	F	SLE	Watery diarrhea for 10 days, vomitting	+	+	+	–
5 TT	38	F	AIDS (heterosexual transmission)	Watery diarrhea for one week, 4–5 times/day	±	±	+	–
6 GG	55	M	AIDS (heterosexual transmission)	Diarrhea for 3 months; 3 times/day	+	+	+	+
7 SY	34	M	NHL	Diarrhea containing mucus for 3 days, 2–3 times/day, vomiting	±	+	+	+
8 EC	56	M	AIDS (transmission by blood transfusion)	Intermittent diarrhea for one year	±	+	+	+
9 VÖ	30	M	AIDS, CMV retinitis (IV drug user)	Watery diarrhea for one month, 3–5 times/day	±	+	+	–
10 ES	45	F	RT	Intermittent watery diarrhea for 2 years, 6–8 times/day	–	–	+	+
11 ÜN	32	F	AIDS (heterosexual transmission)	Watery diarrhea for 6 months, 5 times/day	+	+	+	+

M: Male
F: Female
AML: Acute myeloblastic leukemia
NHL: Non-Hodgkin lymphoma
SLE: Systemic lupus erythematosus
RT: Renal transplantation
BMT: Bone marrow transplantation
PCP: Pneumocystis carinii pneumonia
CMV: Cytomegalovirus
* If structures which resembled but were not exactly typical for the cryptosporidial oocysts were visualized, the result was denoted as ±

Our results indicated the presence of oocysts in fecal samples of all patients with AIDS included in the study by at least one of the methods used. CDC has reported a rate of 3.6% positivity among 19,182 AIDS patients in a survey carried out in 1986. This has been followed by detection of the parasite in 15–60% of AIDS patients, the incidence being notably higher in underdeveloped and developing countries. Laughon et al. [14] detected the parasite in 15.6% of 388 homosexual subjects with diarrhea. Smith et al. [15], on the other hand reported that 3 out of 20 diarrheic AIDS patients were positive for oocysts. The results of another study carried out in Haiti [16] indicated that 11 out of 29 AIDS patients with chronic diarrhea had Cryptosporidiosis. The incidence varied significantly among different countries, being 3% in Denmark, 11% in England, 12% in Brazil, 21.2% in France, 30% in Zaire and 32% in Zambia [17]. The number of AIDS patients is relatively low in Turkey compared to other countries (630 confirmed cases). Although the number of AIDS patients included in our study is low, our preliminary results indicate that the rate of positivity is considerably high. However, since the time for hospitalization and detection of oocysts is close for most of the AIDS patients and the patients were hospitalized in the same ward, it is almost impossible to rule out the existence of nosocomial spread. This high frequency thus was attributed to both a probable nosocomial infection as well as being a similar finding to the relatively high frequencies previously reported from other developing countries.

There have been reports in literature concerning the prevalence of Cryptosporidiosis in organ transplantation patients. Weisburger et al. [18] detected Cryptosporidium in jejunal biopsy specimen of a diarrheic renal transplantation patient taking high dose immunosuppressive therapy. Our study included one renal transplantation patient whose fecal sample was positive for Cryptosporidial oocysts.

Individuals who have underlying neoplastic disorders and are treated with immunosuppressive agents constitute another population with an increased susceptibility to development of opportunistic infection due to Cryptosporidium. A previous study carried out in Turkish patients with neoplastic diseases and gastroenteritis indicated Cryptosporidium oocysts in 16.9% of the study group [19]. 2 out of 9 patients (one acute myeloblastic leukemia and one Non-Hodgkin lymphoma) with neoplastic disorders were found positive for Cryptosporidium in our study. These results indicate that Cryptosporidiosis should be considered to be present in patients with neoplastic disorders.

Laboratory diagnosis of Cryptosporidiosis can be established by various techniques. Differentiation of oocysts and the yeast cells displays difficulties in some instances, which was also the case in our study, particularly during examination of Giemsa stained smears. Those samples were evaluated as positive-negative to avoid over and underestimations. MAF staining method is superior to Giemsa as far as this problem is concerned. However, false positive results were detected by prolonged application of the MAF stain, decreasing the sensitivity of the method [20].

Direct and indirect immunofluorescence tests which use monoclonal antibodies directed against the oocyst wall are known to be sensitive and rapid. Some of the studies showed that immunofluorescence is superior and more sensitive because of the ability of detection of low number of oocysts [8, 9, 21]. However, there have been reports claiming that the morphologic appearance of the oocyst may sometimes be difficult to recognize and nonspecific fluorescence may cause false-positive interpretation [22]. This and the

other factors stated above might be responsible for the differences between the results of direct staining and immunofluorescence methods in our study.

In conclusion, facilities to detect Cryptosporidial oocysts should be carried out in routine parasitology laboratories, particularly for examination of fecal samples of immunosuppressed diarrheic patients. MAF staining method appears to be practical and reliable for this purpose. Immunofluorescence techniques may also be used for confirmation, if possible, since the excreted oocysts may be low in number in some cases.

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